MECHANISMS FOR MAINTAINING STABILITY IN THE HELMETED GUINEA FOWL
NUMIDA MELEAGRIS
WHEN RUNNING ON UNEVEN TERRAIN.

A thesis presented

by

Amanda Hitchcock

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ABSTRACT OF THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology in the Graduate School of Arts and Sciences of Northeastern University, September, 2010
Abstract

Animals must maintain stability when running over rough, uneven terrain, but the methods by which they successfully do so are largely unknown, particularly at high speeds. “Drop-down” studies are a useful way to examine how animals cope with an unexpected change in terrain height. To investigate joint function during and following a drop and make predictions about the likely preflex and reflex responses necessary to compensate for altered mechanical conditions, I ran helmeted guinea fowl *Numida meleagris* over a trackway with a camouflaged drop in substrate height. 2-D inverse dynamics were used to calculate the net moments, powers, and angular trajectory at each joint. The drop dramatically altered limb posture. The ankle, knee and hip all showed significantly increased extension during the drop with differences in angle of 26.5° ± 2.5°, 13.3° ± 3.9°, and 20.4° ± 2.4°, respectively. Net moments at the tarsometatarso-phalangeal joint (TMP) and ankle during the subsequent stance period were reduced but predictable, whereas net moments at the hip and knee were variable and unpredictable, particularly in early stance. Based on these results I predicted reflex alteration of muscle activity at the proximal joints would be necessary to cope with variations in net moment by increasing mechanical impedance. I also predicted muscle shortening of the digital flexors due to ankle extension would reduce force production by these muscles, requiring an increase in muscle activation to compensate and provide enough force to support the stance phase moments. The ankle may require increased activation to maintain the observed extension moment and potentially co-contraction to prevent hyperextension.

To test these predictions, I sampled twelve muscles during both level and drop runs using intra-muscular electromyography (EMG) electrodes. The average EMG amplitude was compared between level and drop runs during 3 time periods: Period 1, the “drop,” the time between
expected foot contact and the average time of actual contact, Period 2, “stance,” the additional period following actual contact during which changes in EMG amplitude would on average be predicted to influence stance phase mechanics, and Period 3, “early swing,” the period of time during which changes in EMG amplitude would be expected to influence the mechanics of pulling the limb from the hole. Two muscles acting at the TMP joint and one acting at the knee/hip showed significant alterations in EMG amplitude during Period 1, indicating that both distal and proximal muscles show the fastest reflex responses. A number of other muscles at the ankle, knee, and hip also showed alterations in muscle recruitment with only a slightly longer delay during Periods 2 and 3. In support of my predictions, increased activation was found in the digital flexors and increased co-contraction was found at the ankle and hip. Increased activation was also found in the knee extensors, likely to compensate for altered contractile conditions. My findings suggest there is a coordinated reflex response across multiple joints in response to an unexpected drop in terrain height.
Acknowledgements

I would like to thank my advisor Dr. Richard Marsh for his endless encouragement, guidance, and advice, and most of all for helping me grow from the lost and overwhelmed student I started as into the person I am now. With his help I’ve learned how to be a better scientist and a stronger person. I would also like to thank my committee, Dagmar Sternad and Joseph Ayers, for their ideas, support, and infinite patience. A huge thanks must also go to all the members of the Marsh lab, past and present, especially to Matthew Propert, Christopher Camara, and Andrew Yegian for the countless hours spent chasing angry birds across trackways. Without all their help I would never have managed to pull this off, and it wouldn’t have been half as fun. I would also like to thank the University for its financial support, and the following grants: National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR47337 and National Science Foundation Grant IOS-0542795. Lastly, a special thank you to my mom for giving me the kickstart I needed to get out there and make something of myself, and for always being there when I need her most.
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<th>Abbreviations</th>
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<tbody>
<tr>
<td>AFD</td>
<td>Actual Footdown</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>EDL</td>
<td>Extensor digitorum longus</td>
</tr>
<tr>
<td>EFD</td>
<td>Expected Footdown</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>FCLP</td>
<td>Flexor cruris lateralis pars pelvica</td>
</tr>
<tr>
<td>FIR</td>
<td>Finite Impulse Response</td>
</tr>
<tr>
<td>FT</td>
<td>Femorotibialis</td>
</tr>
<tr>
<td>GRF</td>
<td>Ground Reaction Force</td>
</tr>
<tr>
<td>IC</td>
<td>Iliotibialis cranialis</td>
</tr>
<tr>
<td>ILPO</td>
<td>Iliotibialis lateralis pars postacetabularis</td>
</tr>
<tr>
<td>ILPR</td>
<td>Iliotibialis lateralis pars preacetabularis</td>
</tr>
<tr>
<td>IP</td>
<td>Interphalangeal joint</td>
</tr>
<tr>
<td>LG</td>
<td>Lateral head of the gastrocnemius</td>
</tr>
<tr>
<td>Mean Drop FD</td>
<td>Mean footdown time in drop runs; 0.027 ms after expected footdown</td>
</tr>
<tr>
<td>MG</td>
<td>Medial head of the gastrocnemius</td>
</tr>
<tr>
<td>n</td>
<td>Sample size</td>
</tr>
<tr>
<td>Post IF</td>
<td>Posterior portion of the iliofibularis</td>
</tr>
<tr>
<td>TC</td>
<td>Tibialis cranialis</td>
</tr>
<tr>
<td>TMP</td>
<td>Tarsometatarso-phalangeal joint</td>
</tr>
<tr>
<td>VO₂ max</td>
<td>Maximal oxygen consumption</td>
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Chapter 1: Alterations in joint function in guinea fowl *Numida melagris* encountering an unexpected drop in terrain height.

Abstract

Animals must maintain stability when running over unpredictable terrain, but the methods by which they successfully do so are largely unknown, particularly at high speeds. To investigate joint function during and following a perturbation and make predictions about the likely preflex and reflex responses necessary to compensate for altered mechanical conditions, I ran helmeted guinea fowl *Numida melagris* over a trackway with a camouflaged drop in substrate height. The response to an unexpected change in substrate height can be described in two phases: Phase 1, the time between expected and actual contact, and Phase 2, the period following actual contact, as the limb contacts the ground in a more extended position than normal. 2-D inverse dynamics were used to calculate the net moments (i.e. torque), powers, and angular trajectory at each joint. The drop dramatically altered limb posture. In Phase 1 of the drop runs, the ankle, knee and hip all showed significantly increased extension with differences in angle of $26.5^\circ \pm 2.5^\circ$, $13.3^\circ \pm 3.9^\circ$, and $20.4^\circ \pm 2.4^\circ$, respectively. Net moments at the tarsometatarso-phalangeal joint (TMP) and ankle were reduced but predictable whereas net moments at the hip and knee were variable and unpredictable. Based on these results I predict reflex alteration of muscle activity at the proximal joints is necessary to cope with variations in net moment by increasing mechanical impedance. At the TMP, muscle shortening of the digital flexors due to ankle extension should reduce force, requiring an increase in muscle activation to compensate and provide enough force during stance. The ankle may require increased activation to maintain the observed extension moment and potentially co-contraction to prevent hyperextension. My findings suggest a coordinated reflex response across multiple joints is
necessary to maintain normal function in an abnormal configuration and achieve dynamic stability. These predictions are tested in a companion study (Chapter 2) measuring muscle activity in antagonistic pairs of muscles.

I. Introduction

Animals in their natural environment must navigate an uneven, unpredictable landscape filled with obstacles and potential pitfalls. Coping with these threats to stability is a basic requirement of terrestrial movement, yet most research has focused on steady-state locomotion over level terrain. However, there is a growing interest in how animals maintain dynamic stability over rough terrain and the control mechanisms that are involved (Daley et al., 2009; Gorassini et al., 1994; Marigold and Patla, 2005; Moritz and Farley, 2004; Shinya et al., 2009). Existing studies of unpredictable terrain have investigated the response of the limb to a variety of unstable conditions that mimic some aspects of those experienced in nature, such as unexpected changes in surface stiffness, changes in floor position after foot-contact, and concealed changes in terrain height. Such perturbation experiments are a useful way to investigate stability in a controlled setting.

Two general mechanisms are available for stabilizing locomotion in response to a perturbation: 1) preflexes, which rely on the inherent mechanical properties of the musculoskeletal system; and 2) reflexes, which result from alterations in muscle recruitment triggered by sensory receptors (Enoka, 2002). Less is understood about the interplay of these mechanisms during running than in walking. The majority of perturbation studies have examined only walking gaits (Gorassini et al., 1994; Shinya et al., 2009; Van der Linden et al., 2007), but walking and running are mechanically different and impose different demands on the system. Because events during high-speed running are rapid and brief in duration, Daley et al. (2006a;
2006b; 2007) hypothesized that there is less mediation by the CNS (reflexes) than in walking, and a greater reliance on the intrinsic mechanical properties of the system (preflexes). Using results from experiments in which guinea fowl were exposed to an unexpected drop in terrain height, Daley et al. (2007) concluded that the responses in the limb result from a proximo-distal gradient in joint neuromechanical control in which distal muscles around the ankle and tarsometotarso-phalangeal (TMP) joints appear to be highly load-sensitive and controlled by rapid feedback, but the proximal muscles at the hip and knee are load-insensitive and controlled in a feed-forward manner. This conclusion was consistent with their quasi-steady state calculations of net joint moments (torques) and work, which were used to indicate the overall mechanical function of the individual joints during the entire stance period. They found the biggest alterations in overall function appeared to occur at the distal joints. However, this evidence for the proximo-distal control model is quite indirect. The possibility remains that maintaining similar function in the proximal joints with a radically different limb configuration following the drop (Fig. 1.1) may require alterations in muscle activity, and, conversely, large alterations in distal joint function could be caused by the altered posture even if the muscles maintained a similar neural activation (Daley et al., 2009). The proposed proximo-distal gradient of control also stands in contrast to results on humans and cats experiencing a drop in substrate height during walking, which show responses in proximal and distal muscles (Gorassini et al., 2004; Van der Linden et al., 2007). Understanding the responses to an unexpected drop in substrate height could benefit from further investigations that examined the detailed time-varying mechanical conditions of the joints during both the drop and stance phases, and the relation of these conditions to the likely roles of the muscles acting at the joints.
In the present study I used 2-D inverse dynamics to examine alterations in joint function in guinea fowl exposed to an unexpected drop in terrain height using a protocol similar to that of Daley et al. (2006a; 2006b; 2007; 2009). The goal of this work was to predict which joints are most likely to require reflex changes in muscle activation to support their function after the perturbation. Guinea fowl are a useful representative biped; their history as a model species, in addition to the ease with which they can be trained, makes them an advantageous species for study. Bipedal running is of interest not only because of its relevance to humans, but because it poses additional challenges to stability due to the fact that only one limb contacts the ground at a time. These challenges are demonstrated by the differing responses of quadrupeds and bipeds to the type of perturbation study used by Daley et al. and others, and which I have used here, commonly known as a “drop-down” study. Drop-down studies provide a convenient and repeatable way to investigate the impact of a sudden unexpected change in terrain height, such as a camouflaged drop in a level trackway containing a hidden force plate. Because the animal is unable to anticipate the drop, it must instead recover by making rapid corrective responses. In walking quadrupeds, e.g. cats, the response is to rapidly pull the foot out of the hole (Gorassini et al., 1994; Heibert et al., 1994). In walking humans, the unexpected step-down triggers a fast reflexive braking reaction in both limbs (Shinya et al., 2009; Van der Linden et al., 2007). In contrast to both of these situations, when the foot of a running biped such as a guinea fowl enters the hole, the other limb is still in swing phase. Because the limb entering the hole is the only support the bird must extend its limb into the hole and cope with the altered limb angles and moments that result.

When the foot fails to make contact with the ground at the expected time, there are two phases involved in the response. Phase 1, the drop, is the time between expected and actual
footdown. Phase 2 is the shortened stance phase following actual footdown. In Phase 2 the limb contacts the ground in a more extended position than normal (Fig. 1.1). Phase 1 has not been addressed before in guinea fowl because joint mechanics were calculated using a quasi-static approach that allowed estimates of joint moments only during stance (Daley et al., 2007). Most human studies also have ignored Phase 1 because either the perturbation was initiated after heel-strike or the drop was so small that there was little change in joint positions during the drop. Examining Phase 1 using inverse dynamics would provide new insight into rapid control mechanisms in effect immediately following a perturbation. The inverse dynamics calculations are used along with information on the muscular anatomy at each joint to predict which muscles are likely to require reflex changes in activity to provide a stable response to the drop.

II. Methods

Seven juvenile helmeted guinea fowl were obtained from a local farmer and trained to run on a treadmill five days a week for half an hour each day at a range of speeds, varying from a walk of 0.67 m/s to a fast run (within VO₂ max) of around 2.5 m/s. Training kept the birds fit and encouraged them to run at a constant speed. The birds were given ad libitum access to food and water. All procedures involving live animals were approved by the Northeastern University Institutional Animal Care and Use Committee. At the time of data collection the birds had a mean body mass of 1.31 ± 0.08 kg.

The track was approximately six meters long and had a coarse rubberized surface (3M™ Safety-Walk™ Medium Duty Resilient Tread 7741) to prevent slipping during runs. A force plate using six-axis load-cell (AMTI model MC3A-6-250, AMTI Inc., Watertown, MA) with a custom built top plate made from aluminum honeycomb was set into the trackway to record the vertical, horizontal, and mediolateral ground reaction forces as the bird made contact. The plate
dimensions were 230 mm by 255 mm. Force data were digitally converted using a Powerlab /16SP controlled by the application LabChart (ADinstruments, Colorado Springs, CO, USA). One wall of the track had a section of clear plastic, which allowed video recording of the bird as it ran across the force plate. The height of the runway could be altered to raise it above the force plate (creating the drop in substrate height). Measurements done on a sample of our guinea fowl found an average standing hip height of 22.5 cm. This was used to determine the height of our drop during perturbation trials, 8 cm (35% of standing hip height, similar to Daley et al., 2006a; 2006b; 2007; 2009).

The location of the force plate was disguised using tissue paper to prevent the bird from seeing the upcoming drop. Concealment was aided by painting the trackway and tissue paper with randomly arranged patches of black and gray; i.e., disruptive coloration. Using a force transducer connected to the tarsometatarsus of a guinea fowl foot, we measured the force required to break the tissue paper. On average this force was 0.36 N for a foot traveling at approximately 2 m/s. This small external force does not appear in our inverse-dynamics calculations. However, assuming an orientation of the force vector at tissue paper contact that is similar to that at level ground contact, the joint moments (torque) that would have been caused by the force required to break the tissue paper were predicted to be less than 10% of the net moments resulting from inertial effects at this time. During experimental runs, drop-down trials were interspersed among level trials (1 drop-down for every 10-12 level runs), to prevent the bird from learning to anticipate the drop and altering its behavior.

When the birds completed training and reached adult body mass, they were given the opportunity to run across the track (without substrate drop) prior to the experiment in order to acclimate them to the room and lighting. Introducing them to the experimental setup in advance
reduced distraction during actual runs. A covered cage was placed at the end of the track. Practice trials showed that most birds quickly became motivated to run towards the cage.

Videography was done using a Mikrotron (Model EoSens CL) camera at a speed of 500 frames per second with a Nikon lens (Model AF-S Nikkor 17-35 mm 1:2.8 D ED). Video files were streamed directly to hard disc using the application StreamPix (Norpix, Inc.) running on a custom configured PC. During each recording session, we videoed a calibration grid placed successively at near and far edges of the track. Data from these calibration grids and the lateral position of the center of pressure on the force plate were used to correct for parallax errors. The birds’ leg and back feathers were clipped prior to track runs and retro-reflective markers were glued to key locations, found by palpating the limb, in order to track the movement of the limb segments as the bird passed in front of the camera. Marked joints included: the toe, interphalangeal (IP) joint, tarsometatarsophalangeal (TMP) joint, the ankle, and two pelvic markers (Fig 1.2). The two pelvic markers were attached at a known distance from the hip joint (verified post-mortem) and used to calculate hip position. The position of the knee was calculated using the positions of the hip and ankle and the lengths of the femoral and tibiotarsal segments (measured post-mortem), as in Donelan et al. (2009) and Rubenson and Marsh (2009). We used the joint coordinate system (Fig. 1.2) specified in Rubenson and Marsh (2009). In this system, positive changes in angle at the ankle, knee, and hip are defined as flexion; negative changes are extension. For the TMP joint, positive increases in angle are defined as digital-extension, and negative increases as digital-flexion. These sign conventions have been used in previous human studies and were chosen so that the sign of the net joint moments during the calculations would be consistent (Rubenson and Marsh, 2009). Marked points were tracked using an auto-tracking plugin MTrack2 (Nico Sturrman) for ImageJ (Wayne Rasband, NIH).
A successful run was one in which the bird moved at approximately constant speed over the force plate and made contact with the force plate only with the foot of the marked leg. Runs that did not meet these criteria were discarded. The force plate data and kinematic data were low-pass filtered in Igor Pro (Wavemetrics, Lake Oswego, OR, USA) using a zero phase-shift binomial smoothing algorithm (Marchand and Marmet, 1983), with a cutoff of approximately 50Hz. The smoothing parameters in Igor Pro to achieve the desired cutoff frequencies were calculated using the formula in the supplementary methods of Mosharov and Sulzer (2005). After processing the video footage in ImageJ, the tracked points were also imported into Igor Pro where they were smoothed and interpolated. The required segment properties were determined on a separate set of birds using methods similar to those of Rubenson and Marsh (2009), except that the body segment included the head and neck. The segment properties were used along with the kinetic and kinematic data to calculate the joint angles, net moments, and net powers, at each joint throughout the stride. The calculations used standard inverse dynamics principles (Winter, 1990) and were implemented in custom functions written for Igor Pro.

Statistics:

Means are given ± 1 standard error of the mean. Statistical comparisons between drop and level groups were done using an ANOVA implemented with the General Linear Model in SPSS v18, with drop vs level as a fixed factor and speed as a covariate. The mean values presented in the results are the means adjusted for speed resulting from the ANOVA.

III. Results

Mean running speed across trials was 2.5 ± 0.12 m/s. Level trials had a mean stance duration of 118 ± 7 ms. Phase 1 (the drop) lasted a mean of 27.7 ± 0.0 ms. Stance phase (Phase
2) in drop trials had a mean value of 70 ± 7 ms. Interestingly, although stance was shortened in drop trials, Phase 1 and Phase 2 together (i.e., Expected Footdown through Toe-off) had a mean value of 97 ± 8 ms and was not significantly different ($P = 0.168$) from the mean stance duration of level running. In level trials the limb performed positive net work during stance, likely due to acceleration across the plate, with a mean net work of 0.37 ± 0.09 J/kg. Net limb work differed significantly following a perturbation (Table 1.1), on average performing -0.08 ± 0.08 J/kg of net negative work, although individual trials varied and the limb sometimes produced positive net work. Similar to Daley et al. (2007), absolute limb work decreased with increasing limb contact angle (defined as the angle formed by the hip, toe, and ground at footdown).

*TMP Joint*

In level runs the TMP joint went through digital extension from footdown through the first half of stance, followed by digital flexion (Figure 1.3). During Phase 1 of the drop, the joint had little angular change for approximately 15 ms—i.e. half the duration of the drop—then went through digital flexion until actual footdown. In contrast, in level runs digital flexion never occurred prior to footdown and did not begin until approximately 40-50 ms after footdown (mid-stance). After actual footdown in the drops the angular trajectory usually resumed digital extension, although there was greater variability in the joint angles throughout stance than during a level run, likely related to variations in foot position at footdown.

Net moments during Phase 1 of the drop were at or close to zero, likely because there was little inertia in the small distal segments. A net digital flexion moment developed within milliseconds of actual footdown (Phase 2) in both conditions, but mean peak digital flexion moment was significantly reduced following a drop, decreasing from level values by 48.4% (Table 1.2). The combination of angles and net moments resulted in a burst of negative power
followed by a burst of positive power (Fig. 1.4). Average peak negative power and peak positive power following a drop were not significantly different from level (Table 1.2), and net work at the TMP following a drop was not significantly different from level running (Table 1.1).

**Ankle Joint**

In level runs the ankle joint extended until footdown, flexed during early stance, and then extended again until the start of swing. During Phase 1 of a drop, when the foot did not make contact at the expected time, the ankle continued to extend when it would normally have begun flexion (Fig. 1.5). Average extension in the drops during Phase 1 was $24^\circ \pm 1.2^\circ$. Flexion at the ankle did not begin in any drop trials until Phase 2. As a result the ankle joint was significantly more extended following a drop (Table 1.2) with an approximately $26.5^\circ \pm 2.5^\circ$ difference between mean ankle angle at actual footdown between drop and level runs.

Before footdown in both drop and level runs a small extension moment was calculated but this moment was small compared to the extension moments during stance. In both types of runs a substantial net extension moment developed after footdown, but the perturbation resulted in a significant reduction of ankle moments throughout stance (Table 1.2). Average peak extension moment decreased following a drop by 57.7%. This reduced moment in combination with altered ankle angles led to reduced negative power and near-elimination of positive power during stance (Fig. 1.4). Mean peak negative power following a drop decreased significantly by 50.7% and mean peak positive power decreased significantly by 78.4% (Table 1.2). The ankle sometimes absorbed and sometimes produced net work during the drop, but on average tended to do more negative work. Mean net work during stance following a drop was significantly reduced by 164.7% (Table 1.1).

**Knee Joint**
In level runs the knee began flexion immediately after footdown, while during a drop, the knee performed similarly to the ankle in most trials and extended throughout Phase 1 (Fig. 1.6). The extra extension resulting from the drop lasted an extra 4-12 ms into Phase 2. Total extra knee extension ranged from 2.5° to 28.8°, with an average extension of 12.8° ± 2.6°. As a result, the average knee angle at actual footdown in drop trials was 13.3° ± 3.9° smaller than average knee angle at footdown in level trials, a significant difference (Table 1.2). During Phase 2 and early swing of drop trials, the knee flexed more rapidly than during the level runs, such that angles were the same between the two types of runs shortly after the onset of swing.

The knee experienced a net flexion moment during level runs that began in late swing (due to inertial effects) and continued through early stance, and was characterized by large and rapid fluctuations in net moment at footdown (Fig. 1.6). During a drop, net moments remained approximately constant during Phase 1. During Phase 2 there was much greater variability in net moment than in level runs, both immediately after footdown and during mid to late stance. Changes in peak net flexion moment and peak net extension moment were not significant (Table 1.2). In level runs, the knee experienced a burst of positive net power at footdown followed by a burst of negative net power (Fig. 1.7). At the end of Phase 1 of perturbed trials there was sometimes a small momentary spike in negative power. Actual footdown was then followed by increased net positive power relative to level runs in early stance, although this increase was not significant. Net peak negative power remained similar between drop and level runs and there was no significant difference. The knee performed nearly equal amounts of negative and positive work in both conditions, resulting on average in little to no net work and no significant difference between drop and level runs (Table 1.1). However, there was considerable variability and net work in each individual run ranged from -0.24 J/kg to 0.43 J/kg.
Hip Joint

The hip joint normally begins extension in late swing and continues extending through footdown and through most of stance (Fig. 1.8). The hip continued to extend throughout Phase 1 of the unexpected drop for an average of 20.4° ± 2.4°. Because extension post-footdown is normal, quantifying the extra amount of extension above that in level runs is difficult. However, I found that the joint was in a significantly more extended position than normal at the initiation of actual footdown (Table 1.2).

The resulting net moments were variable and fluctuated rapidly. Several perturbed runs had a spike of net flexion moment at footdown as well as greater net flexion moment later in the stride. Average peak net extension moment was significantly reduced following a drop by 50.3%, while average peak net flexion moment significantly increased by 50%. The resulting net powers were also much more variable than during level running (Fig. 1.7), although the change in average peak positive power was not significant (Table 1.2). Mean net work at the hip decreased significantly by 60.5% (Table 1.1).

IV. Discussion

When an animal encounters an unexpected drop in terrain height, the mechanical conditions at the leg joints rapidly deviate from expected conditions at foot contact, and the overall response of the limb must be stabilizing if the animal is to avoid a fall. Daley et al. (2006a; 2006b; 2007; 2009) focused extensively on the overall mechanical response. The present study focuses on the individual joints and the predicted mechanisms that compensate for the altered mechanical conditions at these joints.
Changes in the net joint moments due to delayed contact with the substrate and altered joint angles following contact can be accounted for by either reflex alterations in muscle activation, and/or preflex changes in muscle force due to the inherent mechanical properties of the active and passive muscles and other limb structures. In addition to the altered mean moments at the joints, the animal may have to deal with increased variability in these moments. One strategy for dealing with increased variability is a general stiffening of the joints by co-contraction of antagonistic muscle pairs, which may require altered activation patterns.

The balance of interaction between preflexes and reflexes during high-speed running is unknown. Reflex changes in activation are known to play a large role in walking. For example, perturbation studies of walking cats suggest that about 30% of total muscle activity during level walking is due to force feedback (Donelan et al., 2009). Whether these control mechanisms function the same way during high-speed running is uncertain due to the latencies involved in reflexes, and it is thought that intrinsic mechanical factors play a larger role in running than walking, particularly in proximal muscles (Daley et al., 2009). However, this does not rule out the possibility of reflex response. Sophisticated feedback control found at the shoulder and elbow provides clear evidence for complex coordinated reflex responses across joints in a multijoint system (Kurtzer et al., 2008, 2009). Current models of spinal cord control of legged locomotion clearly allow for rapid reflex feedback during the movements of the limb (McCrea, 2001).

In the following discussion, I use the observed net moments and angular trajectories found in this study to make predictions about likely preflex and reflex responses in the muscles producing the moments at each joint. Additionally, I examine the variability in joint moment after ground contact to predict which joints might require increased co-contraction. Measurements of electromyographic activity in level and drop runs will be useful in testing these
predictions. Although I focus on a joint-by-joint analysis, the alterations in mechanical function across the joints are certainly linked.

**Mechanical Conditions at the TMP Joint**

During the drop the TMP joint (Fig. 1.9A) had little change in joint angle, which leaves it at a similar angle during initial ground contact as that found in level running. Daley et al. (2007) similarly found little change in angle had occurred at the TMP joint at footdown following the drop. The digital flexor muscles normally activate in anticipation of the substantial digital flexion moment that develops during stance (Marsh et al., 2004). During Phase 1 the angular change is small and the predicted net TMP moment is near zero. These data indicate that the force in the digital flexors was greatly reduced during Phase 1. During Phase 2 of the perturbed runs a substantial net digital flexion moment develops, although it is significantly smaller than the moment during level runs (Fig. 1.3, Table 1.2). This moment must be supported by rapid force development in the digital flexors.

**Predicted reflex and preflex activity at the TMP joint**

Daley et al. (2007) considered it likely that the muscles operating at the TMP joint would be subject to rapid proprioreceptive feedback during the drop, but also suggested that preflex mechanisms might play a role in altering force in these muscles. I consider it unlikely that the reduced force in the digital flexors during Phase 1 is due to reflex inhibition of motor recruitment to these muscles. The digital flexor muscles have a substantial moment arm at both the ankle and TMP joints (Fig. 1.9A). Because rapid ankle extension occurs during Phase 1 of the drop, the short-fibered digital flexors would likely experience a greatly reduced force compared to that for level runs because of combined force-velocity and length-tension effects. Also, any reflex inhibition of activity in the digital flexors would be unlikely to influence force development in
these muscles until Phase 2, because of reflex and electromechanical delays. Another possible mechanism for reducing the net moment at the TMP joint is passive force due to stretch of the extensor digitorum longus (EDL) resulting from ankle extension. Therefore, I predict that the observed decrease in force during Phase 1 is due to reflex mechanisms involving shortening of the digital flexors and possibly lengthening of the EDL, rather than reductions in muscle activation. In contrast, the conditions in Phase 2 may require an increase in activation of the digital flexors.

Following footdown in the drops (Phase 2), the digital flexors may require increased motor recruitment because they need to produce a substantial digital-flexion moment while operating at shorter lengths. Whether the proprioreceptors in the foot or other sensory systems can respond rapidly enough to alter activation during Phase 1 in preparation for ground contact is not clear, but if so, this preactivation would provide force in early stance, precisely when it is needed. Increased co-contraction during stance is not predicted at the TMP joint because the joint angle is similar to that found in level runs and the moment is predictably in net digital flexion.

*Mechanical Conditions at the Ankle Joint*

In level running, the ankle extends through late swing and begins flexion within milliseconds of foot contact. When the substrate height is lower than expected, the ankle continues extending throughout the drop (Daley et al., 2007; this study) and on average the joint is in a much more extended position (26.5° difference) than normal at the beginning of stance. In late swing in level runs, my calculations indicate a small net extension moment is present at the ankle, and this extension moment increases rapidly after ground contact. When the birds encounter the drop, the small net extension moment found in late swing is maintained through
Phase 1, and then increases rapidly after ground contact reaching a peak value of 50.2% of that found during stance in level running. Thus, the moment data suggest that the force in the muscles producing an ankle extension moment is greatly reduced in Phase 1 and somewhat lower in Phase 2. The major muscles that can produce the required extension moment are the digital flexors and the two large heads of the gastrocnemius, lateral and medial (Fig. 1.9B). These extension moments could be opposed by active or passive force in the tibialis cranialis (TC) and the extensor digitorum longus.

**Predicted reflex and preflex activity at the Ankle Joint**

Daley et al. (2007) hypothesized that the ankle extensors are activated in a feed-forward manner in anticipation of stance, followed by proprioceptive changes in activation after the foot has made contact. However, in a subsequent study, Daley et al. (2009) did not find a significant difference in EMG intensity in the lateral gastrocnemius during stance when comparing drop and level runs, suggesting that alterations in ankle moment are due to preflex mechanisms alone. However, these data do not rule out reflex changes in the medial gastrocnemius or the digital flexors.

In discussing the TMP moments I hypothesized that the force in the digital flexors is likely very low in Phase 1 due to force-velocity and length-tension effects. Low force in the digital flexors will also reduce the net ankle extension moment in Phase 1. The situation for the gastrocnemius muscles is more complex. The lateral gastrocnemius has a substantial flexor moment arm at the knee and its velocity and length in Phase 1 will be a balance of a shortening effect due to increased ankle extension and a lengthening effect due to knee extension. Daley et al. (2009) found that following the drop the fascicles of the lateral gastrocnemius are at a similar length to that found during stance in level runs. The bulk of the fascicles of the medial
gastrocnemius insert on the tibiotarsus and are not affected by knee angle, but some anterior fascicles have a knee extensor moment arm. Thus, the medial gastrocnemius is expected to shorten substantially more during the drop than in level runs, mostly due to ankle extension, but also due to knee extension. Daley et al. (2009) recorded the combined force of all heads of the gastrocnemius during level and drop runs using a tendon buckle on the Achilles tendon. They found that the gastrocnemius force increased in Phase 1, but remained substantially below the value expected during this period in level runs. Because the net ankle moment remains low during this period the force in the Achilles tendon is likely balanced by increasing passive or active force in the tibialis cranialis and the extensor digitorum longus.

In Phase 2, I have predicted an increased activation of the digital flexors to support the digital flexion moment at the TMP joint, and this increased activation would also aid in producing the ankle extension moment following foot contact. Whether increased activation of the heads of the gastrocnemius is also needed is not clear. The medial gastrocnemius is expected to be substantially shorter than the length during level runs, but the lateral gastrocnemius is likely at a similar length based on the findings of Daley et al. (2009).

My results suggest changes in co-activation may not be necessary at the ankle to maintain stability during stance. The net ankle moments were smaller than those found during stance in level runs, but were predictably always in extension. However, the ankle approaches the anatomical limit of extension during the drop, and it is possible that co-contraction may be needed to prevent hyperextension. Human perturbation studies have found that co-contraction increases at the ankle following a perturbation. Nakazawa et al. (2004) found short latency reflex EMG responses (<50 ms) following a 10mm drop in substrate height (after heel-strike) not only in the ankle flexors and extensors, but in the tibialis anterior, which is not normally active during
stance. Their results show that reflexes can activate both swing and stance muscles to increase stability at the ankle.

*Mechanical Conditions at the Knee Joint*

Interpreting the mechanical conditions at the knee based on inverse dynamics is problematical because in normal level running considerable co-contraction is present at the knee (Gatesy, 1999; Marsh et al., 2004). Nevertheless, my results suggest that the mechanical conditions at the knee are altered by the drop. In level runs the knee extended until footdown and then flexed during stance, with a small and variable amount of extension occurring in late stance (Fig. 1.6). The net knee moment in level runs was in flexion in late swing reflecting the inertial forces acting on the segments, and remained in flexion (but with considerable variability) in early stance largely due to the ground reaction forces (Fig. 1.10). As stance proceeds, the net moment transitions to extension with considerable variability in the timing of this transition (Fig. 1.6). The large amount of variability in knee moment during early stance may explain the need for co-contraction to maintain stability during level running (Hitchcock and Marsh, unpublished). During Phase 1 of the drop runs, I found that in most runs knee extension accelerated shortly after tissue paper contact and then continued until after foot contact. The additional extension during stance resulted in a mean minimum knee angle 13° more extended than that during level runs. This result differs from Daley et al. (2007) who found no significant difference in knee angle at ground contact between level- and drop-runs. This difference may be a result of my method of determining knee position, which used hip and ankle positions and segment lengths, rather than marking the knee directly. Calculating knee position avoids errors due to skin movement at the knee (Rubenson and Marsh, 2009). After reaching a minimum angle the knee flexes rapidly during Phase 2. During Phase 1 of the drop the net knee moment remains
in flexion but with increased variability and in Phase 2 following foot contact the mean knee moment remains in flexion before transitioning to extension. However, the variability in net moment during stance is larger than that found in stance during level running. These data largely agree with those of Daley et al. (2007) in that on average the knee has a similar function during stance in drop and level runs. However, my data indicate that this similar function must be maintained in the face of much greater variability in net moment, and with a more extended knee.

*Predicted reflex and preflex activity at the Knee Joint*

Predicting accurately the preflex alterations in force output of the flexors and extensors at the knee is difficult without a complete musculoskeletal model because of the many two-joint muscles which cross the ankle or hip (Fig. 1.9C) and because of co-activation of extensors and flexors. The balance of flexion and extension forces remains approximately the same during Phase 1 of the drop but with greater variability. The major knee extensors active at this time are the posterior iliotibialis lateralis, which is also a hip extensor, and the monoarticular femerotibialis complex. Because the knee and hip both extend during Phase 1, extension force is expected to be lower than during the comparable period in early stance in level runs. A lower extension force with a similar net moment must mean that force in the flexor muscles also must decrease. Active knee flexors include the lateral gastrocnemius, which is also an ankle extensor, and the hamstring-like muscles (posterior iliofibularis, posterior flexor cruris lateralis, and flexor cruris medius), which are also hip extensors (Fig. 1.9). The posterior flexor cruris lateralis also can act as an ankle extensor (Ellerby and Marsh, 2010). Knee extension during the drop would tend to lengthen these knee flexors, but hip extension and ankle extension also occur, which
would tend to shorten them. Detailed knowledge of the instantaneous moment arms of all of these muscles is needed before an accurate prediction of the net effects can be made.

After ground contact in Phase 2 the net moments are highly variable, which likely requires effective co-contraction to produce a stable trajectory of knee angle. Co-contraction is normal at the knee and the knee normally experiences greater fluctuations at footdown than the other joints, suggesting co-contraction is necessary to stabilize the knee and create a predictable moment for the muscles to work against. Fluctuations in net moment are even larger and more unpredictable following a perturbation (Fig. 1.6) and may require increased mechanical impedance, which could be achieved by increased co-contraction.

Mechanical Conditions at the Hip Joint

In most level runs the hip extended from late swing through the end of stance, but in some cases extension was delayed at foot contact and there was minimal angular change during early stance. The net moment is almost always in extension immediately before and during stance, yielding net positive work for the hip. In Phase 1 of the drops the knee extends, resulting in a considerably more extended angle at contact as compared to the value at contact in level runs. Because of the more extended limb the vector of the ground reaction force is much closer to the hip in drop runs (Fig. 1.1), which results in relatively small but highly variable hip moments immediately after contact.

In a normal level run net moment at the hip is predictably in extension until the end of stance. My results agree with those of Daley et al. (2007) who found that the hip is more extended at contact following the drop. Despite the difference in joint angle at the hip the prevailing moment is in extension following the drop and the hip does net positive work. Daley et al. (2007) concluded that the overall joint function appeared to be similar in drop and level
runs, and as a result they hypothesized that the hip extensors act in a primarily feed-forward manner and are insensitive to load. However, my results show that following the drop there were rapid and highly variable fluctuations in net moment at the hip (Fig. 1.8). Peak net extension moments were overall reduced while peak net flexion moments increased, with considerable variation among runs. The observed variability in net moment likely results from different limb orientations following the drop.

Predicted reflex and preflex activity at the Hip Joint

In level runs there is little or no co-contraction at the hip during footdown, which correlates with the extension moments being predictably in extension. However, increased co-contraction may be required following a drop to cope with the large variability in net moment. Thus, even though the overall mechanical function at the hip does not change, I predict that reflex activation of the hip flexors in concert with the extensors (Fig. 1.9D) occurs to increase mechanical impedance and provide needed stability.

V. Conclusions

My results suggest that a simple proximo-distal gradient of control model may not be enough to explain how the limb maintains stability in the face of an unexpected drop in substrate height. Instead, a coordinated reflex response across multiple joints is predicted. Even though the overall function of the proximal joints does not change following the drop, alteration of muscle activation may be required to maintain function with an altered limb posture. At the hip, the most proximal joint, inverse dynamics calculations indicate a large variability in net moment immediately after the limb contacts the ground in a more extended position following a drop. I hypothesize that the hip flexors, which are normally silent during stance, will be activated to
increase mechanical impedance and stabilize the joint. At the TMP, the most distal joint examined, I predict that increased activation of the digital flexors will be required to compensate for reduced force due to muscle shortening caused by ankle extension during the drop. Reflex alteration of activation may also be required at the knee, although predicting the required changes is difficult given that many of the muscles that cross the knee also act at the hip and the ankle. Thus, I predict that a coordinated reflex response throughout the limb will be required to compensate for altered joint mechanics. Evidence that this type of coordinated response is possible comes from human studies. Nakazawa et al. (2004) found evidence of a short-latency response in the rectus femoris, a knee extensor and hip flexor, following a 10 mm drop in substrate height during human walking. In studies of arm movement Kurtzer et al. (2008; 2009) demonstrate evidence of coordinated reflexes that allow feedback from perturbations at distal joints to influence activity of more proximal muscles. They hypothesize that this sophisticated feedback may rely on an internal model of limb dynamics, or, alternately, it may be evidence of direct mapping of sensory inputs and motor outputs across multiple joints. The predictions of the present study relying on inverse dynamics are tested in a companion study in which muscle activity was measured in muscles acting at the various joints in the leg (Chapter 2).
VI. Literature Cited:


Figure 1.1. Position of the limb at footdown in a normal level run (left) and following an unexpected drop in substrate height (right). Stick figures were generated using tracked points from actual runs, then overlaid with a representation of the skeleton and lines of action of selected muscles. The blue vector represents the ground reaction force (GRF) and the blue circle indicates the center of mass.
Figure 1.2. Figure from Rubenson and Marsh (2009). A: A diagram illustrating the coordinate system used to describe angle change at each of the joints. Positive changes at the hip, knee, and ankle are designated flexion; negative changes are extension. The coordinate system of the digits is set at a 90° angle to the other joints; positive changes are termed digital extension and negative changes are digital flexion. B: The limb shown set to a neutral position where all angles are zero. C: A close-up of the coordinate system of the TMP and phalanges.
<table>
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Table 1.1. Mean net work performed during stance in level and perturbed runs for the limb as a whole and at each joint individually. Values with significant changes are in bold. Means are adjusted for speed. N= 12 for both level and drop means.
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Table 1.2. Average angle at actual footdown (AFD), mean peak net moment, and mean peak power at each of the joints in both drop and level runs. Values with significant changes are in bold. Angles at AFD at the TMP are not presented due to uncertainty in precise position of the joint at the instant of footdown caused by the tissue paper obscuring the marker. Peak net extension and/or flexion moments are presented only when applicable (i.e., results for peak net flexion moment are given only if the joint has a net flexion moment), as with peak positive and/or negative net power.
Angular Trajectory and Net Moments at the TMP Joint

Figure 1.3. Angular trajectory and net moment at the TMP joint. Level runs are in red; drop trials are in green. Positive increases in angle and net moment represent digital extension; negative increases indicate digital flexion. All runs are aligned at Expected Footdown (zero), indicated by a vertical line. Mean footdown during drops (Mean Drop FD) is at 0.027ms indicated by a second line. Inverted triangles designate footdown and normal triangles designate toe-off for individual runs. Line type in the top graph indicates speed: Crosses = >3 m/s; solid line = 2-3 m/s; dashed line = <2 m/s.
Figure 1.4. Net power at the distal joints. Level runs are in red; drop trials are in green. All runs are aligned at Expected Footdown (zero), indicated by a vertical line. Mean footdown during drops (Mean Drop FD) is at 0.027ms indicated by a second line. Inverted triangles designate footdown and normal triangles designate toe-off, for individual runs.
Figure 1.5. Angular trajectory and net moment at the Ankle joint. Level runs are in red; drop trials are in green. Positive increases in angle and net moment represent flexion; negative increases indicate extension. All runs are aligned at Expected Footdown (zero), indicated by a vertical line. Mean footdown during drops (Mean Drop FD) is at 0.027ms indicated by a second line. Inverted triangles designate footdown and normal triangles designate toe-off for individual runs. Line type in the top graph indicates speed: Crosses = >3 m/s; solid line = 2-3 m/s; dashed line = <2 m/s.
Figure 1.6. Angular trajectory and net moment at the Knee joint. Level runs are in red; drop trials are in green. Positive increases in angle and net moment represent flexion; negative increases indicate extension. All runs are aligned at Expected Footdown (zero), indicated by a vertical line. Mean footdown during drops (Mean Drop FD) is at 0.027ms indicated by a second line. Inverted triangles designate footdown and normal triangles designate toe-off for individual runs. Line type in the top graph indicates speed: Crosses = >3 m/s; solid line = 2-3 m/s; dashed line = <2 m/s.
Figure 1.7. Net power at the proximal joints. Level runs are in red; drop trials are in green. All runs are aligned at Expected Footdown (zero), indicated by a vertical line. Mean footdown during drops (Mean Drop FD) is at 0.027ms indicated by a second line. Inverted triangles designate footdown and normal triangles designate toe-off, for individual runs.
Figure 1.8. Angular trajectory and net moment at the Hip joint. Level runs are in red; drop trials are in green. Positive increases in angle and net moment represent flexion; negative increases indicate extension. All runs are aligned at Expected Footdown (zero), indicated by a vertical line. Mean footdown during drops (Mean Drop FD) is at 0.027ms indicated by a second line. Inverted triangles designate footdown and normal triangles designate toe-off for individual runs. Line type in the top graph indicates speed: Crosses = >3 m/s; solid line = 2-3 m/s; dashed line = <2 m/s.
Figure 1.9. Lines of action of selected muscles acting at the: A) TMP joint, B) Ankle joint, C) Knee joint, and D) Hip joint. A dashed circle indicates each joint. Muscles that extend the hip, knee, and ankle, or produce digital extension at the TMP joint, are shown in blue, while muscles that flex the hip, knee, and ankle, or produce digital flexion at the TMP joint are shown in red. Muscles referred to in the text are labeled with their abbreviations.
Figure 1.10. Net knee moment in a sample level and drop run calculated using two different techniques. Expected footdown is at zero indicated by a vertical line. Footdown in the drop trial (labeled Drop FD) is designated by a second vertical line. Solid lines indicate results calculated using a complete 2-D inverse dynamics model, including all inertial moments, while dashed lines were calculated using the quasi-static approach (i.e., the force vector approach) and exclude inertial moments.
Chapter 2: Coordinated reflex response in muscles of the guinea fowl hind limb following an unexpected drop in terrain height.

Abstract

Natural environments are composed of rough, unpredictable terrain that animals must navigate in order to maintain dynamic stability. A recent study of joint function in running guinea fowl exposed to an unexpected drop in terrain height hypothesized that reflex alterations in muscle activation are likely necessary to cope with altered mechanical conditions in the abnormally extended limb. To test these predictions, I sampled twelve muscles using intramuscular EMG electrodes in helmeted guinea fowl running over a trackway with a camouflaged drop in substrate height. The average EMG amplitude was compared between level and drop runs during 3 time periods: Period 1, the “drop,” the time between expected and actual contact, Period 2, “stance,” the period following actual contact during which changes in EMG amplitude would on average be predicted to influence stance phase mechanics, and Period 3, “early swing,” the period of time during which the animal pulls the limb from the hole. The FL and supDFIII, two muscles acting at the TMP joint, and the ILPR, a hip flexor/knee extensor, showed significant alterations in EMG amplitude during Period 1, indicating that both distal and proximal muscles show the fastest reflex responses. A number of other muscles at the ankle, knee, and hip also showed alterations in muscle recruitment with only a slightly longer delay during stance and early swing. Increased activation was found in the digital flexors and knee extensors and increased co-contraction was found at the ankle and hip. These results suggest there is a coordinated reflex response across multiple joints in response to an unexpected drop in terrain height.

I. Introduction
Terrestrial animals face numerous threats in their natural environments, but the greatest threat to their stability may in some cases be the environment itself. Natural landscapes are composed of rough, uneven, unpredictable terrain, presenting a continuous challenge to terrestrial locomotion. Although most research has focused on steady-state locomotion over level terrain, there is now growing interest in how animals achieve dynamic stability over rough terrain and the control mechanisms that are involved, especially during high speed running. Progress in this area has been achieved through perturbation experiments, which examine the responses of the locomotor system to unstable conditions that attempt to mimic those experienced in nature, for example, unexpected changes in surface stiffness or terrain height (Daley et al., 2006; Daley et al., 2007; Daley et al., 2009; Gorassini et al., 1994; Moritz and Farley, 2004; Nakazawa et al., 2004). Knowledge of the control mechanisms involved in dynamic stability is important to our understanding of the limb and its function in normal everyday activity, and to our ability to understand and treat locomotor disorders.

Stabilizing responses to a perturbation during locomotion can involve both reflexes and preflexes. Preflex responses are due to the inherent mechanical properties of the active and passive muscles, the multiarticular arrangement of active muscles, and other features of the locomotor system. For example, force-velocity and length-tension effects can allow rapid responses to changes in velocity and length (Enoka, 2002). Also, increased mechanical impedance via co-contracting sets of muscles may allow a joint to withstand rapid fluctuations in joint moment (i.e. torque) by altering the external loads on the limb. Reflex alteration of muscle activity is triggered by various sensory receptors and occurs with variable latencies depending on the sensory systems activated (Enoka, 2002). These receptors include those inside the muscle-tendon unit, such as muscle spindles and Golgi tendon organs (monitoring length change and
changes in force respectively), and cutaneous and vestibular motor receptors. The two mechanisms, preflexes and reflexes, are not mutually exclusive. Reflex activity alters the properties of the muscular components of the limb, thus altering the preflex system. Events during high-speed running are rapid and brief in duration, leading to the hypothesis by Daley et al. (2006; 2007) that there is less mediation by the CNS (reflexes) than in walking and a greater reliance on the intrinsic mechanical properties of the muscle (preflexes).

Guinea fowl are useful representative bipeds and have a history as a model species in the study of bipedal locomotion. As a result they have been used in a variety of studies of both steady-state and unsteady-state locomotion. Earlier research involving an unexpected drop in terrain height, commonly known as a “drop-down” study, revealed a variety of dynamic responses to this perturbation that allow the guinea fowl to recover and continue running (Daley et al., 2006; 2007; 2009). Daley et al. (2006; 2007) proposed that stability following the drop is facilitated by a proximo-distal gradient of neuromechanical control. In this model, muscles acting at the distal joints were hypothesized to be load-sensitive, responding rapidly to mechanical and proprioceptive feedback and altering their mechanical performance using both preflex and reflex adjustments in order to cope with the perturbation. However, proximal muscles were found to have similar overall mechanical function in level and drop runs and were therefore suggested to be load-insensitive and controlled in a feed-forward manner, i.e., perturbations are not expected to lead to reflex alterations in muscle activity.

In the drop-down protocol used by Daley et al. (2006) and also in the present study, using running guinea fowl the expected reflex latency and electromechanical delay in force development make it unlikely that reflex responses can modify the mechanics of the guinea fowl limb during Phase 1, the period of time between expected and actual foot contact. However,
reflexes could play a stabilizing role in Phase 2, the stance phase following the drop. Phase 1 of the perturbations lasts approximately 27 ms (Chapter 1; this study). Daley et al. (2009) reported a transmission delay of about 6 ms for the gastrocnemius stretch reflex using a tendon tap test and suggested a further electromechanical delay of 34 ms for force development. Measurement technique has a significant effect on the estimates of electromechanical delay, for example, delays between activation and force development in the human gastrocnemius have been found to be only 16 ms in the absence of tendon slack (Muraoka et al., 2004). Nevertheless, the total minimum latency in guinea fowl is likely to be only slightly less than the time it takes for the drop. Assuming a minimum latency of approximately 25 ms suggests that reflexes could play a role in stabilizing Phase 2 which typically lasts 70 ± 7 ms following the drop (Chapter 1).

In contrast to the proximo-distal control hypothesis of Daley et al (2007), an inverse dynamics study of guinea fowl running using a drop-down protocol similar to that of Daley et al. (2006; 2007; 2009) led to the hypothesis that a coordinated reflex response involving muscles acting at all of the leg joints is required to allow a stable recovery from the drop (Chapter 1). Substantial extra extension was found at the ankle, knee, and hip prior to actual footdown, leaving the limb in an unnaturally extended position (Chapter 1, Figure 1.1). Distally, reflex activation of the digital flexors was hypothesized to increase force production at short fascicle lengths. More proximally, the perturbation resulted in increased variability in net joint moments during stance at both the knee and hip. Increased co-contraction, including reflex activation of the hip flexors which are normally only active during swing, was thus hypothesized to be necessary to increase stability as the extended limb reacts to the ground reaction forces. The goal of the present study was to use electromyographic (EMG) recordings to test my hypothesis that
during rapid running a coordinated reflex response occurs across joints in response to an
unexpected drop in substrate height.

In this study, I sampled muscle activity from twelve muscles acting at the major joints of
the guinea fowl leg during a drop-down perturbation. In testing for significant changes in EMG
intensity that reflect reflex activity, the null hypothesis was that the timing and intensity of EMG
during and following the drop followed a preprogrammed pattern determined before the expected
contact with the ground and were not influenced by the perturbation. I made comparisons of
EMG intensity in level and drop runs using three time periods. Period 1, “drop,” is the 27 ms
following expected footdown. This period corresponds approximately to the time from expected
foot contact until actual foot contact in the drops, i.e., to Phase 1 (Chapter 1). Given the expected
electromechanical delay, EMG activity during this period would influence the muscle force
development in the earliest part of the stance phase of the drop runs. Period 2, “stance,” is
defined as the time from the end of Period 1 until 25 ms before the time of predicted toeoff based
on running speed (see Methods, Fig. 2.1). This second period corresponds on average to the
additional time after Period 1 during which EMG activity would be expected to influence the
mechanics of stance, assuming a 25 ms electromechanical delay. Period 3, “early swing,” is
defined as the period of time from the end of Period 2 until 25 ms after predicted toeoff. The
time period corresponds to the earliest part of swing phase during which the animal is pulling the
limb out of the hole. We did not examine events later in swing phase because the birds often
clipped the edge of the hole with their foot during extraction and we were concerned about
artifacts due to the vibration of the limb caused by this impact.

II. Methods
Data were collected on six helmeted guinea fowl obtained as juveniles and trained to run on a treadmill five days a week for half an hour each day at a range of speeds, varying from a walk of 0.67 m/s to a fast run (within VO$_2$ max) of around 2.5 m/s. Training kept the birds fit and encouraged them to run at a constant speed. The birds were given *ad libitum* access to food and water. During the data collection period the birds had a mean mass of $1.31 \pm 0.08$ kg. All procedures involving live animals were approved by the Northeastern University Institutional Animal Care and Use Committee.

The birds used to collect EMG data here were also the birds used for inverse dynamics calculations in a companion study (Chapter 1). A portion of the runs used for inverse dynamics calculations were performed by birds after being instrumented with EMG electrodes. No systematic differences were found in the results of the inverse dynamics obtained on instrumented and uninstrumented birds.

*Implantation of the EMG electrodes*

Twelve muscles surrounding the tarsometatarsophalangeal joint (TMP), ankle, knee, and hip were chosen for study (Table 2.1). The large femerotibialis muscle had two electrodes implanted and the results from both muscles were standardized and averaged in trials during which both electrodes gave usable results. Though there are potentially many other limb muscles that may play a role in maintaining stability, we chose major muscles that had actions at all of the joints in the limb. The muscles instrumented include antagonistic muscle pairs at the ankle, knee, and hip.

EMG electrodes with offset hooks (1.5 mm exposed tips, 3.0 mm tip offset) were made from twisted pairs of Teflon-coated stainless steel wire (316SS3T; Medwire). Depending on the intended muscle, the electrodes were 20 cm long (proximal muscles) or 30 cm long (distal
muscles). The electrodes were attached to a custom made lightweight connector that included a braided stainless steel ground wire. The birds were anesthetized with isoflurane (3% to induce, 2-2.5% to maintain) during all surgical procedures and kept warm with a heating pad. The EMG electrodes were routed from an incision on the dorsal pelvis to incisions near the muscles and were inserted into the muscles parallel to the fascicles using chamfered 25G needles. The ground wire remained under the skin on the dorsal pelvis. The connectors were anchored to the pelvis with suture and all incisions closed with 4-0 polypropylene suture. An analgesic (1 mg/kg of butorphanol) was administered before the birds recovered from the anesthetic. The birds were then allowed to rest and recover overnight before beginning experimental runs. At the completion of all trials the birds were sacrificed and the location of the electrodes was confirmed during dissection.

**Experimental setup for the running trials**

The trackway used was identical to that described in Chapter 1, except a taut steel wire was suspended approximately 1 meter above the track to support the weight of the EMG cables that ran from the connector on the pelvis of the bird to the recording system. The cables were loosely held to the steel wire via a sliding ring and allowed movement across the track with minimum interference from dragging the cable. The force required to pull the cable, determined by pulling the cable manually at the approximate speed the birds ran, was 0.4 N.

**Signal processing**

EMG signals were preamplified (1000x) using DAM 50 preamplifiers with analog high pass and low pass filters set to 10 and 3000 Hz, respectively. Signals from the preamplifiers were digitally converted using a PowerLab /16SP and the application Chart (ADInstruments). The signals were then processed using Igor Pro (Wavemetrics). In Igor Pro the EMG signals were
filtered with a custom designed FIR filter, with a bandpass of 90 – 1000 Hz. Signals were then rectified and a “linear envelope” calculated using a binomial smoothing algorithm with a cutoff frequency of 30 Hz (Fig. 2.2). The mean amplitudes of the linear envelopes across specified periods of time were used to compare level and drop runs.

Normalization of signal amplitude

Recordings took place in a number of sessions spanning 2-4 days depending on the animal. I found significant changes in the EMG amplitude for most muscles across the recording sessions. Signals were normalized to the amplitude in the first recording session for each bird by measuring the mean amplitude of the level running stride immediately before the experimental stride and averaging across all runs within a recording session. The EMG recordings of the same muscle in different birds also had differences in amplitude, but these differences were accounted for in our statistical analysis. EMG duration and amplitude was also influenced by speed. To avoid sample bias equal numbers of drop and level runs were analyzed for each animal using pairs of drop and level runs matched for speed.

Calculation of the time periods for comparing EMG amplitudes

Our goal in choosing the time periods for comparison was to avoid sample bias by selecting equal time periods following footdown in level runs and expected footdown in the drop runs, using speed matched trials. This method tests the null hypothesis that the pattern and intensity of EMG found in level runs is unaltered by the drop. Period 1, the 27 ms following expected foot down, was chosen to test whether any muscles alter their activation during Phase 1 of the drop runs before the foot contacts the lowered surface. Period 2 was chosen to encompass on average the portion of the stance phase in the drop runs during which alterations in muscle activation could be expected to influence stance phase mechanics. Assuming a 25 ms
electromechanical delay, this period ended 25 ms before the predicted toeoff time. The combined
duration of the Phase 1 and 2 in the drop runs as a function of speed was approximately equal to
the stance duration in level runs (Chapter 1), except for a small number of runs from two of the
six birds, which had very high limb contact angles after the drop and very short contact times
(Fig. 2.1). These few runs apparently exhibited an extreme form of the vertical kinetic energy
mode of Daley et al. (2006). Excluding these runs, I predicted toeoff from the combined
regression of stance duration in level runs and the sum of drop and stance duration in drop runs.
Although over a large range of speeds stance duration has a curvilinear relation with speed
(Gatesy, 1999a), a linear model fit the data well over the speed range used. Period 3 started at the
end of Period 2 and extended 25 ms after predicted toeoff, corresponding to early swing phase in
level and most drop runs.

Statistics

Mean amplitude of the linear envelope for each EMG trace was calculated for each of the
three time periods. All statistical analyses were performed using SPSS v18 (SPSS Inc., Chicago,
IL, USA). To remove variation in EMG amplitude among individual birds and the variation
correlated with speed we first ran an ANOVA (General Linear Model in SPSS) with bird as a
random factor and speed as a covariate. The standardized residuals saved from this analysis had
equal variance and were used to test for the effect of drop versus level using a second ANOVA.
After examining the average traces we re-ran the same analysis, splitting Period 2 into 2 equal
time periods. Sample size varied across bird and muscle due to the behavior of the birds and the
failure of some electrodes. Table 2.2 indicates the sample sizes.

III. Results
Sample average linear envelopes for individual runs are given in Figure 2.3 for each muscle. These samples provide an example of the variability observed among trials and birds. Although the statistics were run on the residuals after removing the effects of speed, we also present averaged traces across all birds in Figure 2.4 to provide a sense of changes in average amplitude. To produce these average waves we first averaged separately the drop and level linear envelopes for each individual bird after normalizing the time scale to the average duration of the three time periods examined statistically. We then normalized for the traces for individual birds using the mean value across all three phases in level runs and averaged these normalized traces across birds.

*Period 1 (“Drop”)*

The FL, supDFIII, and ILPR showed altered muscle activation during Phase 1 (Table 2.3, Fig. 2.4). These differences in activation were not large compared to changes found in other muscles but were highly significant. Average EMG amplitude increased over level by 51% in the FL, 41% in the supDFIII, and 33% in the ILPR. The average amplitude at expected footdown matched the average amplitude at actual footdown for Stride 1 in drop runs (the level step prior to the drop) and both Stride 1 and 2 of level runs; i.e., there were no changes in activation prior to expected footdown that would suggest anticipation was an influence (Fig. 2.3, Fig. 2.4). However, as Phase 1 progressed all three muscles showed rapid changes in activation.

*Period 2 (“Stance”)*

Muscles with significant increases in mean EMG amplitude calculated for all of Period 2 following a drop include the FT, FL, TC, ILPR, and IC (Table 2.3, Fig. 2.4). For the FT, five out of six birds showed a consistent pattern of greatly increased activation (164% increase over level) after actual footdown in drop trials. Average EMG amplitude in drop runs increased over
the value in level runs by 71% in the FL and 83% in the TC. Several birds were found to have extremely large bursts of activity in the ILPR, resulting in an average increase over normal level amplitude of 580% for this muscle. Similarly, the IC showed variable activation and had large bursts in a few birds, resulting in an average increase over level of 249%.

Due to its anatomical location (deep to the FL), the TC is difficult to implant and can be dislodged easily. Because of this we had successful recordings from only three birds. These three birds showed consistent changes in EMG activity in Periods 2 and 3, but clearly some caution is warranted in interpreting these results.

The EMG amplitudes for the FCLP and ILPO were not significantly different between drop and level runs when the values were calculated for all of Period 2. However, after examining the averaged linear envelopes (Fig. 2.4), I observed that in these muscles the mean EMG activity in drop runs was higher than that in level runs only during the second half of Period 2. Therefore, I repeated the statistical analysis after splitting Period 2 into two equal time periods. I found the EMG increase in amplitude for the second half of Period 2 in drop runs to be significantly different from the value in level runs for both the ILPO and FCLP ($P = 0.007$ and $P = 0.02$, respectively). Splitting Period 2 did not influence the conclusions regarding any other muscle.

Period 3 (“Early Swing”)

A statistically significant increase in muscle activation during early swing was found in the LG, TC, FL, posterior IF, ILPO, FCLP, FCLA, ILPR, and IC. An extra burst of EMG activity in the LG occurred in most drop trials at the initiation of swing phase (Fig. 2.3, Fig. 2.4). The magnitude of this burst varied between trials and birds, with an average increase over level amplitude of 335%, and it always occurred approximately at toeoff. Definite increases in EMG
amplitude above the values in level runs also occurred in the TC (50%), ILPR (110%), IC (41%), and the postIF (240%). Although the mean amplitudes of the FL, ILPO, FCLP, and FCLA were all significantly different from the level values in Period 3, the characteristics of the signals in Period 3 suggest that they were contaminated by crosstalk (Fig. 2.5), and the significant differences should be viewed with caution. The likely source of increased crosstalk in drop runs for the ILPO, FCLP, and FCLA was the large burst in EMG activity in the postIF, and for the FL crosstalk could be from the TC, which is just deep to the FL in the anterior shank.

### IV. Discussion

Although the following discussion takes a joint-by-joint approach, this should not be taken to imply that we ascribe to a muscle-by-muscle neural control model for guinea fowl locomotion. Other research suggests neural control may rely on flexible combinations of groups of muscles activated as units, called “muscle synergies,” which would greatly simplify the task of controlling movement (Tresch and Jarc, 2009). Common muscle synergies have been found in walking, swimming, and airstepping in postembryonic chicks, although the recruitment order, timing, and duration of activity of the muscles within a synergy varied (Johnston and Bekoff, 1996). Johnston and Bekoff (1996) suggested that motor control during these movements is organized around common synergies but that within a synergy muscles can be differentially affected by afferent inputs and muscle activity can be modified on an individual basis. My data are compatible with the idea of muscle synergies, although it is not possible to identify synergies within my results and doing so is outside the scope of the present study. My data are also consistent with evidence that afferent feedback to the central pattern generator (CPG) has a coordinated effect on multiple parts of the system (McCrea, 2001), although most existing CPG
models are based on mammals and not birds. Bradley and Bekoff (1990) found coordinated muscle activation during spontaneous motility in chick embryos and have suggested there is a basic lumbosacral network involved in controlling rhythmic limb movements. In the absence of any modulation (i.e., floating in amniotic fluid inside the egg) this network produces symmetric, alternating activation of the flexors and extensors across several joints. The idea of synergies is fully compatible with current models of the CPG in that interneurons in the spinal cord are proposed to activate specific populations of motor units that innervate synergistic groups of muscles (McCrea and Rybak, 2008). This study does not attempt to address the underlying neural circuitry involved in motor control and instead focuses on alterations in joint mechanics following a perturbation and the corresponding reflex responses.

*TMP Joint*

The two muscles with a digital flexor action at the TMP joint, supDFIII and FL (Fig. 2.6A), showed significant changes in activation during Phase 1 and the EMG amplitude of the FL was also significant in Phase 2. Although in some situations the FL acts as an ankle extensor, at the combination of ankle and TMP joint angles found in both level and perturbed runs, it is predicted to act almost solely as a digital flexor (M. Propert and R.L. Marsh, unpublished). The activation levels at expected footdown matched those of actual footdown in level runs, i.e., there was no evidence of anticipation affecting the motor pattern. In Chapter 1, I predicted that the force in the digital flexors is greatly reduced during Phase 1 of a perturbation, followed by a substantial (though reduced) increase in force during stance. The reduction in force during Phase 1 was predicted to be due to preflex alterations in force due to length-tension and force-velocity effects. However, reflex activation of the digital flexors was predicted to be necessary to maintain a normal level of force during Phase 2 because the muscles operate at shorter lengths.
The increased activity of the FL and supDFIII in Periods 1 and 2 support this hypothesis of reflex activation to produce force during stance.

*Ankle Joint*

In both level and perturbed runs the ankle experiences a large net extension moment immediately at footdown (Chapter 1). The major muscles that can produce this moment are the lateral and medial heads of the gastrocnemius and the digital flexors, which have an extensor moment arm at the ankle (Fig. 2.6B). Therefore, enhanced activation of supDFIII (and possibly other digital flexors from which we did not record) would aid in the production of the needed extension moment during stance. The timing of the increase in activity in the digital flexors likely allows force development at the ankle to begin almost immediately at footdown. Although the LG and MG did not have any changes in activation in Periods 1 or 2, recordings of muscle force from tendon buckles on the Achilles tendon of guinea fowl (Daley et al., 2009) indicate that these muscles produce force in stance phase of drop runs, but at a reduced level compared to the stance force in level runs.

Although net joint moments at the ankle during stance are predictably in extension, I hypothesized in Chapter 1 that co-contraction may be needed to prevent hyperextension. The significant increase in EMG amplitude of the TC in Period 2, the major ankle flexor, supports this hypothesis, although caution is warranted because the TC was successfully recorded in just three birds (Table 2.2).

*Knee Joint*

Co-contraction is normal at the knee joint at footdown and increased co-contraction following a perturbation may be expected to cope with the greater variability in net moments, especially in the period immediately after actual footdown (Chapter 1), although testing this
hypothesis is made more difficult because co-contraction at the knee is a normal feature of activation in level running. Generalized increases in activity of flexors and extensors did not occur early in Period 2. None of the knee flexors showed significantly increased activation during the first part of Period 2, although the ILPO and FCLP had significant increases in activity late in Period 2 (Fig. 2.4). It is difficult to accurately predict the balance of forces at the knee without a more complete musculoskeletal model due to the many two-joint muscles that also cross the ankle or hip (Fig. 2.6C).

Selective activation of the knee extensors was not predicted in Chapter 1, but in the present study I found significant increases in activity during Period 2 in the FT, a monoarticular knee extensor, and in the ILPR and IC, both hip flexors/knee extensors. A significant increase in activity was also found during Period 1 in the ILPR. In hindsight, increased activation of knee extensors may be required because of reduced force output of the FT related to the contractile conditions following the drop. The FT muscle-tendon unit normally lengthens in level runs while active during early stance; i.e., the muscle is active while the joint is flexing. Although this stretch could be absorbed by the tendon, a study using turkeys (Roberts et al., 2007) found 12% lengthening of the FT fascicles in stance during level running. In the drop runs, when the foot does not make contact at expected footdown (EFD) the knee continues to extend throughout Phase 1 and the beginning of stance. Subsequently, the knee angles remain below that found in level runs throughout most of stance. Thus, rather than lengthening during activation, the FT shortens. The fascicles of the FT are approximately 20 mm long and given the moment arm of the patellar tendon at the knee (Carr, 2008), I predict that the fascicles of the FT could be as much as 20% shorter in early stance following a drop than the length at the beginning of stance in level runs. Thus, the force producing capacity of the FT is likely substantially reduced due to a
length-tension effect. Increased activation of the knee extensors, particularly the FT, could compensate for this deficit. Increases in activation in the ILPR and IC are also predicted to have a functional role at the hip.

*Hip Joint*

Evidence of reflex alterations of muscle activation in concert with the predictions of Chapter 1 were also found at the hip. Little or no co-contraction occurs at the hip at footdown during level running. However, because of the very extended limb, net moments at footdown are highly variable following a drop and in Chapter 1 I predicted that co-contraction of hip flexors and extensors may be needed to help stabilize the joint. My results support this prediction, because significant increases in activation occurred during Period 1 & 2 in the ILPR and during Period 2 in the IC, two hip flexors (Table 2.3, Fig. 2.4). The hip extensors maintained normal muscle activation during Period 2, except for the FCLP (a hip extensor/knee flexor) and ILPO (a hip extensor/knee extensor), which increased activation in late stance. Evidence of reflex activation at the most proximal as well as the most distal joint suggests a coordinated reflex response throughout the limb in response to the delayed foot contact occurring in the drop runs.

*Early Swing: All Joints*

Significant changes in EMG activity were found in Period 3, but assigning a functional role to all of these changes is difficult. My earlier study of joint mechanics in drop runs (Chapter 1) did not consider swing phase. One simple hypothesis is that activity of swing phase muscles should increase following the drop in order to rapidly pull the limb from the hole. In concurrence with this hypothesis, I found increased activity in Period 3 in the TA, IC, and ILPR, all muscles normally active during swing phase. However, leaving aside muscles possibly contaminated with crosstalk in Period 3, I also found significant increases in EMG amplitude in the LG and postIF,
which are normally active only in stance (Marsh et al., 2004). The function of these increases in activity is not clear. The anterior portion of the IF is normally active in early swing in level running, and the activity in the postIF could be interpreted as assisting the anterior IF in knee flexion. However, the postIF has a larger moment arm at the hip than the anterior IF and will thus be working against the hip flexors. The LG is an ankle extensor and knee flexor normally active in early stance. In the vast majority of drop runs it had a burst in Period 3, approximately at the stance-swing transition (Fig. 2.3). Several sample recordings given by Daley et al. (2009) also showed a small LG burst near the start of swing. Interestingly, in many runs in which ankle angle was also tracked the rate of flexion slows briefly following toeoff (Chapter 1), which could be due to the Period 3 burst in the LG. However, we do not have enough simultaneous recordings of EMG and ankle angle to make a definite association. The LG also has a substantial flexor moment arm at the knee and the increased LG force in early swing could also aid knee flexion. Any functional significance of this burst of EMG in the LG in terms of responding to the drop is uncertain.

*Sensing lack of foot contact*

When the foot does not meet the ground at the expected time the joints rapidly deviate from the angles and moments expected in level runs. My study did not address the sensors responsible for the reflex changes noted, but my results appear to rule out simple stretch reflexes based on muscle spindles as the only mechanism for triggering changes in muscle activation. For example, the considerable extension undergone by the ankle during the drop is expected to stretch the TC, which could explain its activation in Period 2, but three other muscles showing increased activation in drop runs during either Period 1 or 2, the supDFIII, FL, and FT, are predicted to shorten during the drop and their activation cannot be explained by a simple stretch
reflex. However, stretch receptors in selected muscles could play an important role in the overall reflex response. In humans, van der Linden (2009) found that fast conducting sensory nerves from the lower leg were necessary for the reflex response to delayed foot contact, and these sensory fibers could be from either spindle or cutaneous receptors. Other human studies indicate that stretch in one muscle can elicit reflex responses in muscles that are not stretched (Kurtzer et al., 2008, 2009).

Based on information from other studies, it seems likely that other types of receptors play a role in the response. Cutaneous receptors play a role in sensing foot contact and in regulating locomotor activity during walking (af Klint et al., 2009; Enoka, 2002; McDonagh and Duncan, 2002) and cutaneous receptors that can modulate limb movement are present in birds (Muir and Steeves, 1995). In humans the fastest cutaneous afferents are expected to require only 20 ms to reach the spinal cord from the foot (McDonagh and Duncan, 2002). Limb length in birds is considerably shorter and in general small animals such as guinea fowl are expected to have faster reflexes. Otolith-spinal reflexes could also play a role. Short-latency otolith-dependent reflexes in the cat gastrocnemius occur as early as 10 ms into a sudden fall (Watt, 1976). Visual input plays a large role in locomotion, especially when negotiating obstacles, (Drew et al., 2008) and it is possible that as the birds step into the hole and the head descends the change in optic flow could trigger reflexes, although the latencies that would be involved are unknown. Force sensors also have been found to play an important role in level walking cats, with force feedback accounting for about 30% of total muscle activity in the MG (Donelan et al., 2009). Hiebert et al. (1999) similarly found that in the absence of ground support muscle activity in the knee and ankle extensors of decerebrate level walking cats was reduced to 70% of normal. Loading of the muscle restored activity, suggesting that loss of feedback from the Golgi tendon organs and
primary muscle spindles were primarily responsible for the decrease, although the relative contribution of each is uncertain. In walking humans the contribution of different sensory organs varies throughout the step cycle. Positive force feedback plays a greater role in late stance while spindle afferents likely have a more significant effect during midstance (af Klint et al., 2009). The sensory systems involved in control of locomotion are thus both complex and flexible and more than one sensory system may be involved in the response.

V. Conclusion

A coordinated reflex response throughout the limb.

Previous work on the guinea fowl limb has suggested a proximo-distal gradient in neuromechanical control in which the distal muscles are highly responsive to proprioreceptive feedback but the larger proximal muscles are largely insensitive to it. I found reflex responses at both the most distal and the most proximal joints during Phase 1. I also found reflex responses in numerous other muscles acting at the ankle, knee, and hip with only a slightly longer delay. Therefore, a simple proximo-distal gradient of control is not sufficient to explain the reflex responses to a drop in substrate height, and instead a coordinated reflex response throughout the limb is required. A proximo-distal gradient in neuromechanical control was hypothesized partly because there was no apparent change in joint function at the hip and knee following the drop. Rapid alterations in function at the distal joints are expected to trigger rapid reflexes in muscles acting at these joints, hence, in the absence of any alteration in joint function the proximal joints were not expected to have rapid reflex responses. However, changes in activation may be necessary in order to maintain similar joint function when the limb is in an abnormal
configuration (Chapter 1), and regardless of its source, sensory information may trigger a coordinated response throughout the limb.

Work in other model systems clearly indicates that a multijoint system can have a reflex response extending across all of the joints. In a study of human landing movements, McDonagh and Duncan (2002) had results consistent with the hypothesis that the CNS creates an internal model of pre-set expectations for the sensory results of an action and this model is used to rapidly reset reflex gains. The absence of expected footdown when passing through a false floor triggered reflex responses within 50 ms in both lower limb and thigh muscles. In walking humans Van der Linden et al. (2007) demonstrated a functionally relevant muscle synergy in response to a mismatch between expected and actual footdown that included short-latency activation of ankle, knee, and thigh muscles in both limbs. Kurtzer et al. (2008; 2009) demonstrated that there is sophisticated and flexible feedback control at the shoulder that reflects the integration of sensory information from both shoulder and elbow muscles. Long-latency reflexes at the shoulder could be triggered by elbow displacement alone, without stretching the shoulder muscle. These results applied both during postural control and during perturbations applied during reaching movements, a skilled motor task, which may suggest that long-latency reflexes possess an internal model of limb dynamics, although the pathways responsible for such a model have not been found. Alternately, the same results could be achieved by direct mapping of sensory inputs and motor outputs, where some elbow muscle afferents project to motor neurons controlling the shoulder muscles. Our results showing rapid reflex responses throughout the hindlimb of guinea fowl in response to an unexpected drop fits well with these other data hypothesizing coordinated multijoint reflex responses to the perturbation of the limb.
VI. Literature Cited


Fig. 2.1. Stance duration in level runs (red circles) and combined drop and stance duration in drop runs (green circles and triangles). The triangles represent the combined drop and stance duration for a small number of runs with very high leg contact angles, i.e., the vertical kinetic energy mode of Daley et al. (2006). The black line is the regression through the combined data excluding the data represented by the triangles.
Table 2.1. List of instrumented muscles. The nomenclature used for the muscles is from from Vanden Berge and Zweers (1993). Additional anatomical information specifically for galliform birds can be found in Hudson et al (1959) and Gatesy (1999b).

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Abbreviation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius lateralis</td>
<td>LG</td>
<td>ankle extensor/knee flexor</td>
</tr>
<tr>
<td>Gastrocnemius medialis</td>
<td>MG</td>
<td>ankle extensor</td>
</tr>
<tr>
<td>Tibialis cranialis</td>
<td>TC</td>
<td>ankle flexor</td>
</tr>
<tr>
<td>Fibularis longus</td>
<td>FL</td>
<td>ankle extensor/toe flexor*</td>
</tr>
<tr>
<td>Iliotibialis lateralis pars postacetabularis</td>
<td>ILPO</td>
<td>hip extensor/knee extensor</td>
</tr>
<tr>
<td>Iliotibialis lateralis pars preacetabularis</td>
<td>ILPR</td>
<td>hip flexor/knee extensor</td>
</tr>
<tr>
<td>Iliotibialis cranialis</td>
<td>IC</td>
<td>hip extensor/knee extensor</td>
</tr>
<tr>
<td>Flexor cruris lateralis pars pelvica</td>
<td>FCLP</td>
<td>hip extensor/knee flexor/ankle</td>
</tr>
<tr>
<td>Flexor cruris lateralis pars accessoria</td>
<td>FCLA</td>
<td>extensor</td>
</tr>
<tr>
<td>Iliofibularis, posterior portion</td>
<td>postIF</td>
<td>hip extensor/knee flexor</td>
</tr>
<tr>
<td>Femerotibialis</td>
<td>FT</td>
<td>knee extensor</td>
</tr>
<tr>
<td>Flexor perforans et perforatus digiti III</td>
<td>supDFIII</td>
<td>Flexes digit III</td>
</tr>
</tbody>
</table>

*At the joint angles normally found in drop and level runs, the FL is expected to act only as a toe flexor.
Figure 2.2. Example illustrating the processing of the EMG signals. A. The unfiltered EMG signal. B. The signal after applying the 90-1000 Hz FIR filter. C. The filtered signal after rectification (thin gray line, left axis), and the linear envelope of the rectified signal (thick black line, right axis). The EMG signal was recorded from the femerotibialis muscle in a level stride at 3.5 m s$^{-1}$. Zero time indicates foot contact.
Table 2.2. Sample sizes \((n)\) by bird and muscle. The \(n\) in each case is the total number of runs, including an equal number of speed-matched drop and level runs.

<table>
<thead>
<tr>
<th>Bird</th>
<th>MG</th>
<th>LG</th>
<th>D1III</th>
<th>FL</th>
<th>TC</th>
<th>IF</th>
<th>ILPO</th>
<th>FCL</th>
<th>FCLA</th>
<th>IC</th>
<th>ILPR</th>
<th>FT</th>
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<tbody>
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<td>14</td>
<td>14</td>
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</tbody>
</table>
Figure 2.3. Sample linear envelopes for each muscle. Examples are individual runs taken from different birds. The time scale is normalized to the average duration of the three time periods examined. Black designates recordings from level runs; red designates recordings from drop runs.
Fig. 2.4. Linear envelopes averaged across all birds. The time scale is normalized to the average duration of the three time periods examined. Black designates mean amplitude from level runs; red designates mean amplitude from drop runs. The difference in amplitude between drop and level is shaded for the periods of time during which a significant difference in amplitude was found between the drop and level runs. In two muscles, FCLP and ILPO, the mean EMG amplitude for drop runs was significantly different for the second half of Period 2 but not the first half and this difference is indicated by the hatched area.
<table>
<thead>
<tr>
<th>Muscle</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG</td>
<td>0.788</td>
<td>0.754</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MG</td>
<td>0.137</td>
<td>0.809</td>
<td>0.877</td>
</tr>
<tr>
<td>TC</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>0.015</td>
</tr>
<tr>
<td>FL</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ILPO</td>
<td>0.377</td>
<td>0.007</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ILPR</td>
<td>0.009</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IC</td>
<td>0.781</td>
<td>&lt;0.001</td>
<td>0.008</td>
</tr>
<tr>
<td>FCLP</td>
<td>0.788</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FCLA</td>
<td>0.679</td>
<td>0.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>postIF</td>
<td>0.2</td>
<td>0.553</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FT</td>
<td>0.108</td>
<td>&lt;0.001</td>
<td>0.217</td>
</tr>
<tr>
<td>supDFIII</td>
<td>&lt;0.001</td>
<td>0.544</td>
<td>0.463</td>
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</table>

Table 2.3. Results of the ANOVA analyses comparing the mean EMG amplitude between drop and level runs. P values < 0.05 are taken to be significantly different and are shown in bold. The italicized values for the ILPO and FCLP indicate the P values comparing the second half of Period 2 only.
Figure 2.5. Example of potential crosstalk in a drop run at 3.5 m s\(^{-1}\). Zero time corresponds to expected footdown. Signals are shown from the posterior portion of the iliofibularis muscle (postIF) and the nearby posterior iliofibularis lateralis pars postacetabularis (ILPO) and flexor cruris lateralis pars pelvica (FCLP). All three muscles had EMG bursts at expected foot down. The postIF shows a second EMG burst in the period corresponding to Period 3 (solid box). However, the corresponding activity in the ILPO and FCLP (dashed boxes) is of lower amplitude and frequency than the stance burst in these muscles, and could be crosstalk from the large burst in the postIF.
Figure 2.6. Lines of action of selected muscles acting at the: A) TMP joint, B) Ankle joint, C) Knee joint, and D) Hip joint. A dashed circle indicates each joint. Muscles that extend the hip, knee, and ankle are shown in blue, while muscles that flex the hip, knee, and ankle, or produce digital flexion at the TMP joint are shown in red.